

Use of poly(lactic acid) amendments to promote the bacterial fixation of metals in zinc smelter tailings

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Abstract

The ability of poly(lactic acid) (PLA) to serve as a long-term source of lactic acid for bacterial sulfate reduction activity in zinc smelter tailings was investigated. Solid PLA polymers mixed in water hydrolyzed abiotically to release lactic acid into solution over an extended period of time. The addition of both PLA and gypsum was required for indigenous bacteria to lower redox potential, raise pH, and stimulate sulfate reduction activity in highly oxidized smelter tailings after one year of treatment. Bioavailable cadmium, copper, lead and zinc were all lowered significantly in PLA/gypsum treated soil, but PLA amendments alone increased the bioavailability of lead, nickel and zinc. Similar PLA amendments may be useful in constructed wetlands and reactive barrier walls for the passive treatment of mine drainage, where enhanced rates of bacterial sulfate reduction are desirable.

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1. Introduction

Sulfate-reducing bacteria are indigenous to most soils and sediments (Postgate, 1984; Widdel, 1988). In the course of using a limited number of low molecular weight organic compounds that can support their growth, these anaerobic bacteria use sulfate as a terminal electron acceptor and produce sulfide as a by-product. Increasing amounts of sulfide in the environment lower the redox potential (Eh) and tend to sequester specific heavy metals as relatively insoluble metal sulfides. The tendency of a metal to form a sulfide is a function of its solubility product, and metals such as bismuth (Bi_2S_3 ; $K_{\text{sp}} = 1.6 \times 10^{-72}$), mercury (HgS ; $K_{\text{sp}} = 3 \times 10^{-53}$), silver (Ag_2S ; $K_{\text{sp}} = 1 \times 10^{-51}$), and copper (Cu_2S ; $K_{\text{sp}} = 2.5 \times 10^{-50}$), readily form very insoluble metal sulfides (Ehrlich, 1990). Other heavy metals, such as lead, cadmium, tin, nickel, zinc and iron also form sulfides with low solubilities.

The ability of sulfate-reducing bacteria to generate sulfide has been exploited in biological systems designed to treat and precipitate heavy metals. These applications

range from the use of bioreactors for the precipitation and recovery of metal sulfides (Hammack et al., 1994; Hulshoff et al., 2001), to treatment systems that intercept metal-contaminated groundwater with reactive barrier walls (Benner et al., 1997), to constructed wetlands for the treatment of surface mine drainage (McIntire et al., 1990). When bioreactors are used, conditions that limit bacterial sulfate reduction are carefully controlled. In passive treatment systems, however, in situ microbial activity is usually limited by the concentration of reactive substrates (organic carbon, sulfate, and basic nutrients) and conditions that otherwise limit the optimal metabolic activity of the bacteria (e.g. temperature, pH, oxygen, and metal toxicity). Passive treatment systems are constructed using a limited amount of degradable organic material (such as compost) to support bacterial sulfate reduction activity, so the lifetime of these systems is limited unless additional organic material is supplied (Tarutis and Unz, 1994). Enhancement of bacterial sulfate reduction activity in constructed wetlands for mine drainage treatment has been attempted by directly adding more labile soluble substrates, such as lactic acid and cheese whey (McIntire et al., 1990; Stark, 1991; Hammack and Edenborn, 1992; Christensen et al., 1996). However, this approach is limited by problems related to the design

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and maintenance of substrate delivery systems, the transport and supply of substrate to field sites, and excessive concentrations of dissolved organic carbon in treatment system effluent.

Poly(lactic acid) (PLA) is a biodegradable aliphatic polyester that is finding increased use as a replacement for petroleum-based polymers in the manufacture of packaging materials, synthetic fibers, and other products. The material is produced from lactic acid, which can be generated by the biological fermentation of renewable resources such as starch and sugars. Lactic acid then goes through oligomerization and dimerization steps to form a cyclic diester lactide, followed by its polymerization as the PLA polymer (Jacobsen et al., 2000). In addition to the use of renewable resources as a feedstock, an advantage of such polymers is their ability to be composted and degrade in soil over time. In the case of PLA polymer, this degradation appears to occur initially by abiotic hydrolysis (Kulkarni et al., 1971), resulting in the release of lactic acid, which can be readily metabolized by soil microorganisms. The direct degradation of PLA polymer by microorganisms has also been reported (Torres et al., 1996; Suyama et al., 1998). In the present paper, the potential for exploiting lactic acid released by degrading PLA polymer to drive a bacterial process (sulfate reduction) beneficial in the remediation of a metal-contaminated soil was investigated.

2. Methods

2.1. Site description and sampling

The site is located in Harrison County, WV, where a vertical retort, continuously distilling-type smelter processed zinc ore concentrates between 1910 and 1971. Slab zinc, zinc dust and ball anodes were produced, with an annual capacity of 20 million-kg slab zinc in 1950 (US Bureau of Mines, 1951). In later years, the smelter processed zinc drosses, ashes and other residues to produce zinc dust, oxides and other products (US Bureau of Mines, 1985). Residues taken from the retorts over the lifetime of the smelter produced tailings piles covering 20 ha and up to 30 m high. Past studies have revealed adverse impacts of tailings pile leachates on local groundwater and the West Fork River adjacent to the site (US EPA, 1977). Surface soil was collected from the upper 10 cm of exposed tailings within 100 m of the primary smelter site. The sample was thoroughly mixed in a plastic tub by hand, passed through a 2-mm sieve and kept at 4 °C prior to additional processing.

2.2. Tailings analysis

Particle size determination and total metals analyses were made on tailings that were oven-dried at 105 °C for

24 h. Tailings color was determined using soil color charts (Munsell Color Co., 1994). Particle size distribution was determined by a modified hydrometer method (Soil Texture Unit 1067; LaMotte Co., Chestertown, MD). Effective cation exchange capacity (CEC_e) was calculated based on the sum of exchangeable cations (Ca, Mg, K, and Al) extracted from 2 g oven-dry tailing samples shaken for 2 h with 20 ml 0.1 M BaCl₂ solution (Hendershott and Duquett, 1986). Samples for metals analysis were digested in a 1:1 (vol/vol) mixture of conc. HCl and HNO₃, brought to constant volume, and analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES) on a Perkin Elmer Optima 3000 Radial-View spectrometer.

Water-soluble metals and sulfate in tailings were determined by shaking triplicate samples of 10 g (wet weight) tailings in 100 ml deionized water at 150 rpm on a gyratory shaker for 1 h at room temperature. The supernatant was passed through a 0.45 µm syringe filter and analyzed for trace metals by ICP-OES or sulfate by USEPA method 375.4 (SulfaVer turbidimetric/colorimetric method 8051; Hach Co., Loveland, CO). The reporting limit for the sulfate assay is 0.1 mg sulfate/g soil.

2.3. PLA polymers

Three DL-lactic acid polymers and descriptions of their physical and chemical properties were provided by Cargill-Dow LLC (Minnetonka, MN). The proprietary polymers varied in terms of molecular weight (low or high) and percent residual lactide (low or high). The polymers were in a granular form: 100% of particles were <2 mm, 31% >1.2 mm, and the remainder <0.85 mm in diameter. PLA1 was a low molecular weight (ca. 60,000), high residual lactide (ca. 2%) polymer; PLA2 was a high molecular weight (ca. 176,000), high residual lactide (ca. 2.1%) polymer; and PLA3 was a high molecular weight (ca. 176,000), low residual lactide (ca. 0.2%). The polymers contained a high percentage of the D-lactic acid isomer (17.5–20%), which results in lower crystallinity and greater susceptibility to hydrolysis.

2.4. Laboratory studies

2.4.1. PLA dissolution

PLA polymers were tested for their rates of hydrolysis in aqueous solution. Fifteen grams of each polymer were added to a 1-liter Erlenmeyer flask containing 500 ml of sterile deionized water. Flasks were sealed with Parafilm wrapping film to prevent water evaporation and were shaken at room temperature on an orbital shaker for 224-d. Thirty five milliliters of sample was removed periodically and was replaced with an equal volume of sterile deionized water. L-lactic acid was analyzed with a YSI 2700 Biochemical Analyzer (YSI Inc.,

Yellow Springs, OH) using an L-lactic acid oxidase membrane (YSI 2329) and a calibration standard of 5.0 mM L-lactic acid (YSI 2327). Membrane integrity was tested before each analysis using potassium ferrocyanide solution (YSI 2363). pH was measured using an Orion SA270 portable field meter and a combination pH electrode using a two point buffer calibration (Thermo Orion, Beverly, MA).

2.4.2. PLA-tailings amendment study

One hundred-gram aliquots (wet weight) of tailings were placed in sterile 473 ml capacity glass jars. Tailings samples then received either 5 g polymer (PLA1, PLA2 or PLA3) only; or 5 g polymer (PLA1, PLA2, or PLA3) plus 5 g granular gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$; Baker Refractories, York, PA). Control sediments received 5 g of sterilized 3-mm diameter glass beads. Sterile deionized water was added until the tailings were saturated and there was approximately 1 cm of standing water above the tailings surface. The contents of each jar were then mixed thoroughly. The jars were sealed with Parafilm and canning jar lids, and incubated at 25 °C in the dark. Over the next 656-d, pH and Eh of the tailings were measured periodically. Electrodes were placed directly in the submerged tailings after thoroughly homogenizing the samples with a sterile spatula by hand under a nitrogen atmosphere. Measurements of pH were made using a portable field meter as described previously. Redox potential was determined with an epoxy-body redox platinum combination electrode (Thermo Orion) and was reported in mV relative to the normal hydrogen electrode, after calibration with an equimolar ferri-cyanide–ferrocyanide solution (precision ± 10 mV).

2.4.3. Bioavailable metals

Bioavailable metals in tailings after 656-d incubation were estimated using the modified physiologically based extraction technique (PBET) of Yang et al. (2001). A 100:1 (vol/wt) ratio of 0.011 N HCl (pH 2) to wet tailings was shaken in a 250 ml flask for 1 h at 150 rpm at 37 °C. The solution was taken up in a 10 ml plastic syringe and passed through a 0.2 μm syringe filter.

All ions were determined by ICP-OES using US EPA method 200.7. The percent recovery of calibration check standards typically had a standard deviation $<2\%$ or less per element. The US EPA quality control guideline of one duplicate, one standard recovery, and one spike recovery for every 10 analyses was employed (US EPA, 1983).

2.4.4. AVS:SEM determination

The diffusion method of Brouwer and Murphy (1994) was used to determine the amount of acid volatile sulfide (AVS) and simultaneously extracted metals (SEM) present in tailings samples. A tailings sample (1–2 g wet weight) was placed in a 20 ml scintillation vial (Fisher

Scientific, Pittsburgh, PA) under nitrogen and was immediately frozen at -20 °C and held up to 3-d prior to analysis. A smaller vial (4 ml autosampler vial; Kimble Glass Inc., Vineland, NJ) containing 3 ml nitrogen-sparged sulfide antioxidant buffer solution (SAOB; 2 M NaOH, 0.1 M ascorbic acid, and 0.1 M disodium EDTA) was placed inside the scintillation vial to act as a sulfide trap. Two ml of 2 N HCl were added to the scintillation vial containing the frozen sediment under nitrogen, and the vial was immediately capped. All vials were then placed on a rotary shaker at 150 rpm for 1 h at room temperature. The contents of each inner vial were removed by pipette and diluted in 6 ml additional SAOB solution. The sulfide ion concentration was measured with a sulfide ion selective electrode (Orion 94-16) coupled with a double-junction reference electrode (Orion 90-02). A sulfide stock solution of 1 M was prepared with freshly washed $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ crystals, stored at 4 °C, and used to prepare dilutions in SAOB over the range 10^{-1} to 10^{-5} M for electrode calibration. Sulfide concentrations in stock solutions were independently determined by iodometric titration with 0.025 M sodium thiosulfate. The tailings slurry remaining in each vial was passed through a 0.45 μm syringe filter (Teflon PTFE; Nalgene) and was analyzed for SEM by ICP-OES.

2.4.5. Sulfate-reducing bacteria counts

The most probable number (MPN) of sulfate-reducing bacteria in tailings experiments was estimated by adding ca. 0.5 g of wet tailings to a tube containing 9 ml of anaerobic API sulfate broth (Difco Laboratories, Detroit, MI). One-tenth serial dilutions of this suspension were prepared in triplicate and incubated at 26 °C for 28 d. The 3-tube MPN values were determined from statistical tables (Rodina, 1972) based on the formation of a black FeS precipitate as a positive indication of the presence of sulfate-reducing bacteria. This probability-based method only suggests the order of magnitude of viable bacteria present in the samples.

2.4.6. Statistics

Significant statistical differences between the mean values of bioavailable metals extracted from treated soils were determined by one-way ANOVA with Dunnett's post test using the software GraphPad InStat version 3.05 for Windows 95 (GraphPad Software, San Diego, CA).

3. Results

3.1. Smelter tailings characteristics and metals analysis

The smelter tailings were classified as sandy clays based on particle size analysis (ca. 43% sand, 5% silt, and 52% clay) and were yellowish brown (10YR 5/8;

Munsell Color Co., 1994). The effective cation exchange capacity (CEC_e) was $2.3 \text{ cmol}(+)/\text{kg}$. The water soluble sulfate concentration in the tailings was $2.3 \text{ mmol SO}_4/\text{kg}$ dry weight. Water soluble metals included Zn ($1530 \text{ } \mu\text{mol}/\text{kg}$ dry weight), Mn ($18 \text{ } \mu\text{mol}/\text{kg}$ dry weight), and Cd ($8 \text{ } \mu\text{mol}/\text{kg}$ dry weight). No other water-soluble heavy metals were detected. Total metal concentrations after *aqua regia* digestion are shown in Table 1. Tailings contained high concentrations of total iron and zinc, as well as elevated levels of manganese and other heavy metals (Cd, Cu, Ni, and Pb).

3.2. PLA polymer hydrolysis

Two of the three PLA polymers tested produced free L-lactic acid and decreased the pH of the deionized water medium with time (Fig. 1). PLA1 (low MW, high residual lactide) released lactic acid most rapidly and in the greatest concentration, and PLA2 (high MW, high residual lactide) released lactic acid more slowly. The third polymer tested, PLA3 (high MW, low residual lactide), did not release any detectable lactic acid or change the solution pH (data not shown). Because subsequent experiments with PLA3 gave results similar to controls, those data are not presented. The polymers contained as much as 20% D-lactic acid isomer, so the L-lactic acid measurements reported here may underestimate the total lactic acid concentration in solution. However, selected replicate samples analyzed for total lactic acid by high performance liquid chromatography gave the same patterns of dissolution (data not shown). The results appear to be consistent with the most rapid release of lactic acid from polymers containing initially high percentages of residual lactide, with more rapid hydrolysis occurring as well for the lower molecular weight polymer.

3.3. Effect of PLA and gypsum treatments on tailings characteristics

The changes in tailings pH and redox potential during 656-d incubation in PLA- and gypsum-treated tail-

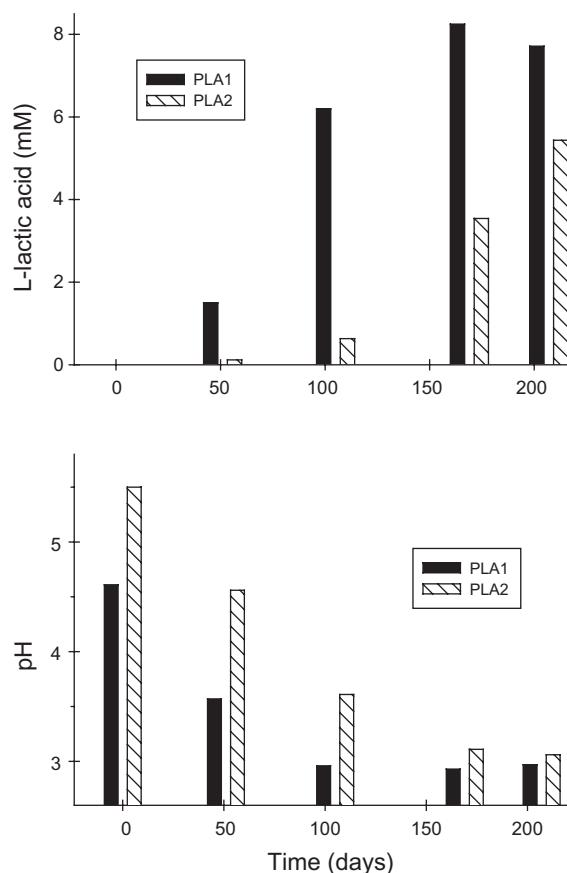


Fig. 1. Release of L-lactic acid and effect on solution pH of 2 different PLA polymer granules shaken in deionized water at 150 rpm at room temperature. Polymers tested included low molecular weight-high percent residual lactide (PLA1), and high molecular weight-high percent residual lactide (PLA2). A high molecular weight-low percent residual lactide polymer (PLA3) did not release detectable L-lactic acid or change the pH significantly and is not shown.

ings are shown in Fig. 2. Initial addition of the slightly alkaline gypsum amendment to smelter tailings raised the pH by about 1 unit, consistent with the poor pH buffering expected from the low cation exchange capacity ($2.3 \text{ cmol}(+)/\text{kg}$) of the tailings material. Little additional change in tailings pH occurred over time after treatment with either gypsum alone or in combination with PLA polymers. In contrast, the addition of PLA1 polymer or PLA2 polymer alone lowered tailings pH after an initial lag period, consistent with the release rate of lactic acid seen in abiotic experiments (Fig. 1). PLA1, the more readily hydrolyzed of the two polymers (Fig. 1) lowered the pH of tailings more rapidly than did PLA2. The pH of unamended control tailings remained relatively constant during the experiment.

Redox potential was dramatically lowered in tailings where PLA polymers and gypsum were both added, but not when gypsum or PLA polymers alone were added. This agreed well with the presence of elevated populations of sulfate-reducing bacteria (SRB) in PLA-gypsum treatments, as estimated by the MPN method. After

Table 1

Total metal concentrations in zinc smelter tailings in $\mu\text{mol}/\text{kg}$ dry weight ($x \pm \text{SD}$, $n = 10$)

As	164 ± 78
Ba	627 ± 84
Cd	115 ± 20
Cr	407 ± 70
Cu	353 ± 200
Fe	461,000 ± 96,000
Mn	5170 ± 1490
Ni	268 ± 60
Pb	323 ± 110
V	709 ± 135
Zn	32,960 ± 8600

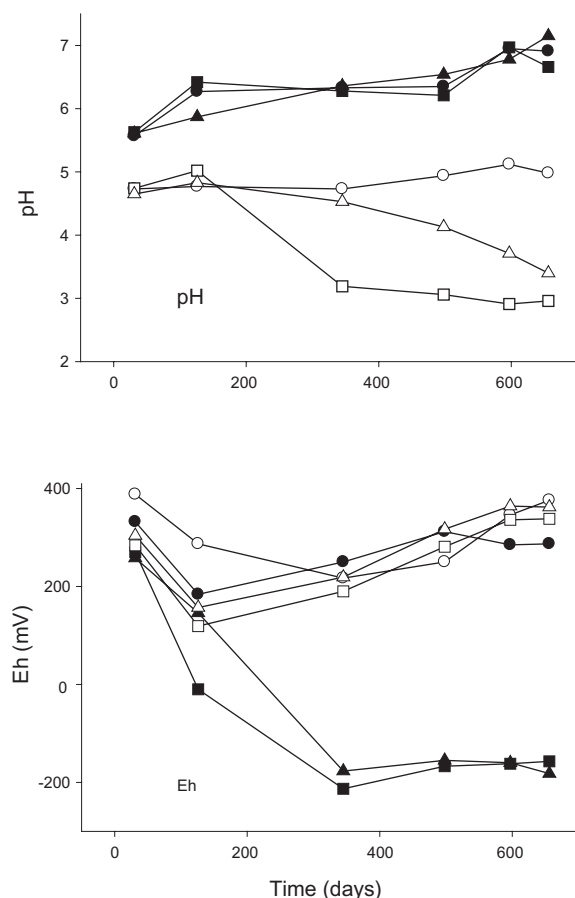


Fig. 2. Changes in pH and redox potential with time for water-saturated zinc tailings treated with PLA polymers and gypsum at 25 °C. Symbols represent no addition (open circle), gypsum only (solid circle), low MW-high percent residual lactide polymer (PLA1; open squares), PLA1 plus gypsum (solid squares), high MW-high percent residual lactide polymer (PLA2; open triangles), and PLA2 plus gypsum (solid triangles).

656-d, SRB ($x \pm SD$, $n = 3$) were only detected in tailings receiving PLA1 polymer plus gypsum ($5.5 \pm 7.3 \times 10^6/\text{gdw}$) and PLA2 polymer plus gypsum ($9.5 \pm 17 \times 10^3/\text{gdw}$). These two values were not significantly different from one another based on the unpaired Student's *t*-test of log-transformed data (two-tailed *P*-value = 0.581).

3.4. Bioavailable metals

PLA and gypsum treatments had various effects on the concentrations of bioavailable metals in zinc tailings after 656-d of incubation. Tailings receiving both gypsum and PLA polymer significantly lowered the concentrations of bioavailable Cd, Cu, Pb, and Zn (Fig. 3). In addition, PLA polymer treatment alone resulted in the formation of greater amounts of bioavailable Pb, Zn, Ni and Fe relative to control tailings (Fig. 3). Iron was generally not bioavailable in control tailings, but was made considerably more bioavailable by the addi-

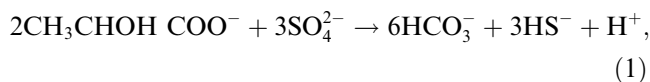
tion of PLA polymers, either with or without additional gypsum amendment.

3.5. Simultaneously extracted metals:acid volatile sulfide ratio

SEM and AVS concentrations in treated tailings after 656-d incubation are shown in Table 2. These data show that significant accumulation of AVS only occurred in tailings treated with both PLA polymer and gypsum, and that neither amendment alone was sufficient to stimulate significant bacterial sulfate reduction in the tailings. Greater than 97% of the SEM pool of heavy metals in the tailings consisted of zinc.

4. Discussion

The addition of both hydrolyzable PLA polymer and gypsum was required to stimulate bacterial sulfate reduction in oxidized zinc tailings and promote the precipitation of bioavailable heavy metals. Based on a generic reaction for the complete oxidation of lactic acid by sulfate-reducing bacteria (Widdel, 1988):



the total amount of sulfide generated by bacteria will be limited by the available lactic acid and sulfate. Bacterial sulfate reduction in tailings required the addition of both of these reactants (Fig. 3 and Table 2). Approximately 347 μmol lactic acid and 520 μmol of sulfate would minimally be required to generate the amount of total AVS detected in PLA-gypsum treated samples in this experiment (Table 2; ca. 520 μmol AVS/100 g). Since approximately 230 μmol of water-soluble sulfate was available, exogenous sulfate generated from solubilized gypsum was required. Making the liberal assumption that three times more lactic acid would have been released from PLA after three times the incubation period (Fig. 1), 120 μmol lactic acid would have been released abiotically in each tailings sample (given ca. 50 ml porewater volume). However, this would not have provided sufficient labile carbon to account for the total amount of AVS produced in the treated tailings.

Two explanations may account for the production of additional lactic acid from added PLA polymer. First, rather than degrading via surface erosion from the surface inward, PLA degrades by bulk erosion, where water slowly plasticizes the polymer, accompanied by a large reduction in the molecular weight and mechanical strength of the material (Griffiths, 2000). Thus, additional release of soluble substrate might have occurred due to the ultimate structural failure or further fragmentation of added PLA polymer at some point during

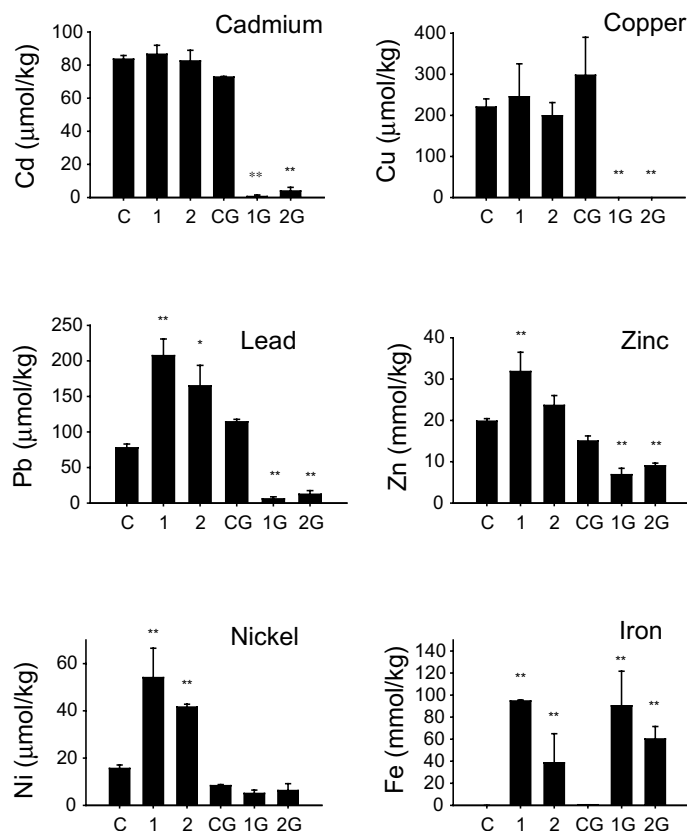


Fig. 3. Bioavailable cadmium, copper, lead, zinc, nickel and iron in PLA- and gypsum-treated zinc tailings after 656-d incubation at 25 °C. Mean \pm SD ($n = 5$) values for samples receiving no addition (C), gypsum only (CG), PLA1 only (1), PLA1 plus gypsum (1G), PLA2 only (2), and PLA2 plus gypsum (2G) are shown. Where shown, single and double asterisks represent significant differences from the control tailings value at the $P < 0.05$ and $P < 0.01$ levels, respectively. Note differences in scale for zinc and iron concentrations.

Table 2

Concentrations of simultaneously extracted metals and acid volatile sulfide in zinc tailings treated with PLA polymers and gypsum and incubated for 656-d at 25 °C ($\bar{x} \pm$ SD, $n = 3$)

Tailings treatment	Simultaneously extracted metals (\sum SEM; $\mu\text{mol/gdw}$) ^a	Acid volatile sulfide (AVS; $\mu\text{mol/gdw}$)	SEM:AVS ratio
Control	18.3 ± 1.1	0.13 ± 0.1	141
PLA1	19.9 ± 0.4	0.2 ± 0.3	100
PLA2	17.5 ± 0.5	0.1 ± 0.04	350
Control + gypsum	15.8 ± 1.1	0.1 ± 0.01	527
PLA1 + gypsum	16.9 ± 1.4	58 ± 3	0.3
PLA2 + gypsum	17.7 ± 1.4	46 ± 9.5	0.4

^a >97% of \sum SEM (Cd, Cu, Pb, Ni, and Zn) was Zn.

the 656-d incubation period of the tailings samples. Second, although abiotic hydrolysis of PLA has been implied as the principal mechanism for its degradation, studies have identified soil microorganisms capable of degrading PLA (Torres et al., 1996). Bacteria and fungi with PLA-degrading capabilities do not appear to be abundant in soil, but they are able to hydrolyze PLA, apparently via the excretion of hydrolytic protease and lipase enzymes, as well as a unique PLA depolymerase (Nakamura et al., 2001). Therefore, more rapid degra-

dation of PLA by indigenous microorganisms in tailings may also have provided additional lactic acid to sulfate-reducing bacteria.

Even when carbon and sulfate sources were supplied to the zinc smelter tailings, a relatively long time was required to generate sulfate-reducing conditions. Briefly, numerous factors could account for this observation, either singly or as a group. Highly oxidized tailings (Fig. 2) would logically undergo a very gradual titration of the oxidized minerals by the small amounts of sulfide

produced as microniches containing active sulfate-reducing bacteria became established and more prevalent. The number of sulfate-reducing bacteria initially present in oxidized smelter tailings would be expected to be low, although they have been detected in most environments investigated (e.g. Postgate, 1984; Widdel, 1988), including copper–zinc mine tailings (Fortin and Beveridge, 1997). Once a viable sulfate-reducing bacterial population had been established, its activity could be slowed by the release of heavy metals initially bound to iron and manganese oxides, or by metals complexed and kept in solution by the lactic acid released from the PLA polymer (Charlatchka and Cambier, 2000). In addition, continued PLA polymer hydrolysis in the absence of bacterial sulfate reduction activity created more acidic conditions with time (Fig. 2), probably due to the accumulation of unmetabolized lactic acid. The lower pH induced by this process would tend to keep heavy metals in solution (Fig. 3), promote metal-organic complexation, and limit the activity of sulfate-reducing bacteria (Rieuwerts et al., 1998). Recent studies examining the composting of PLA polymers with yard waste have demonstrated that the inclusion of too much PLA (>30%) significantly lowered compost pH and suppressed microbial activity (Ghorpade et al., 2001).

The decrease in the relative bioavailability of metals in zinc smelter tailings when bacterial sulfate reduction activity was enhanced is consistent with the known solubility products for the respective sulfides, i.e. $\text{Fe} > \text{Ni} > \text{Zn} > \text{Cd} > \text{Pb} \gg \text{Cu}$ (Ehrlich, 1990). Thus, weak acid-extractable copper, cadmium, and lead appear to have been converted preferentially to their insoluble metal sulfides over nickel and iron in this experiment (Fig. 3). Despite the persistence of bioavailable zinc in tailings after 656-d incubation, this metal represented the greatest sink for bacterially produced sulfide over that time period. The large increase in iron bioavailability after either combined PLA/gypsum treatment or PLA treatment alone (Fig. 3) could be attributable to any of several mechanisms not specifically examined in this study. Direct reduction of ferric oxides by bacterially produced hydrogen sulfide could have been responsible when both amendments were added, whereas low pH (Fig. 2) and organic complexation may have contributed to the observed increase when PLA alone was added. These latter mechanisms are likely responsible for the observed increase in bioavailable lead, nickel and zinc in tailings treated with PLA alone as well. If actual solubilization of iron oxides occurs *in situ* due to one or more of these processes, the potential release of other toxic elements that tend to be strongly bound to iron oxides (e.g. arsenic) must also be considered (Sadiq, 1997).

The potential for metal toxicity in sediments is frequently assessed by examining the molar ratio of the

sum of simultaneously extracted metals (SEM) and acid volatile sulfide (AVS). When the SEM:AVS ratio < 1 , an absence of direct heavy metal toxicity (due to the formation of insoluble sulfide minerals) is suggested. This conclusion is tempered by the understanding that other natural ligands in addition to AVS can serve as binding sites for cationic metals (Sundelin and Eriksson, 2001), that the hydrochloric acid extraction of AVS may release more metals from sediments than are truly bioavailable (Chen and Mayer, 1999), and that sediment toxicity may be due to variables other than heavy metals (O'Connor et al., 1998). Nevertheless, the basis of the AVS:SEM approach to metal toxicity in sediments appears relevant to many different sediments (Chen and Mayer, 1999; Finlayson et al., 2000; Simpson et al., 2000), and this approach proved applicable to the evaluation of the metal toxicity of PLA-gypsum-treated tailings in the current study (Table 2).

The hydrolysis of PLA polymers is influenced by such factors as their molecular weight, crystallinity, percentages of D- and L-isomers, and the formation of copolymers with other alpha-hydroxy acids, such as glycolic acid. This suggests that PLA polymers used for long-term environmental remediation could be better “designed” to provide a sustained release of lactic acid over a long period of time. Poly(lactic-glycolic acid) copolymers (PLGA) have been used in the field of medicine as bioresorbable synthetic sutures, in bone surgery, and as drug delivery systems (Li et al., 1990) due to their ability to degrade to nontoxic products *in vivo*. An apparent advantage of PLGA copolymer over PLA polymer is the greater degree of control over the hydrolysis of the material by varying the percentages of lactic acid and glycolic acid monomers (Griffiths, 2000). Additionally, sulfate-reducing bacteria capable of using glycolic acid have been described (Friedrich et al., 1996). However, the production of these copolymers is currently limited to specialized medical applications and they are unlikely to be generated in large quantities as waste materials suitable for remedial applications in the near future.

5. Conclusions

This study has demonstrated that the addition of sparingly soluble PLA polymer and gypsum to metal-rich smelter tailings could support the activity of indigenous sulfate-reducing bacteria, resulting in the selective precipitation of heavy metals as sulfide minerals. The degradation of specific polymers can be used to uniquely provide a long-term sustained source of monomer constituents that serve as bacterial substrates. New polymers and copolymers continue to be developed whose degradative byproducts could be used in similar novel

applications. As the industrial use of renewable biodegradable polymers such as PLA becomes more common, and disposable waste products generated by this industry become more abundant, the applied use of these materials to facilitate environmental remediation efforts is feasible.

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